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WORDS (WITH MUSIC) ABOUT THE PHARMACIST WHO CONCERTIZED

"Running my pharmacy," he thought, "is like conducting an orchestra. I give the old downbeat and in marches the merchandise. Every item at a price . . . like drums beating.

"I pay for it . . . with a song.

"I sell at a big profit . . . that's a beautiful melody by the entire ensemble."

He waved a baton—but nothing much happened that you could call music. And suddenly he realized he was completely off key.

Moral

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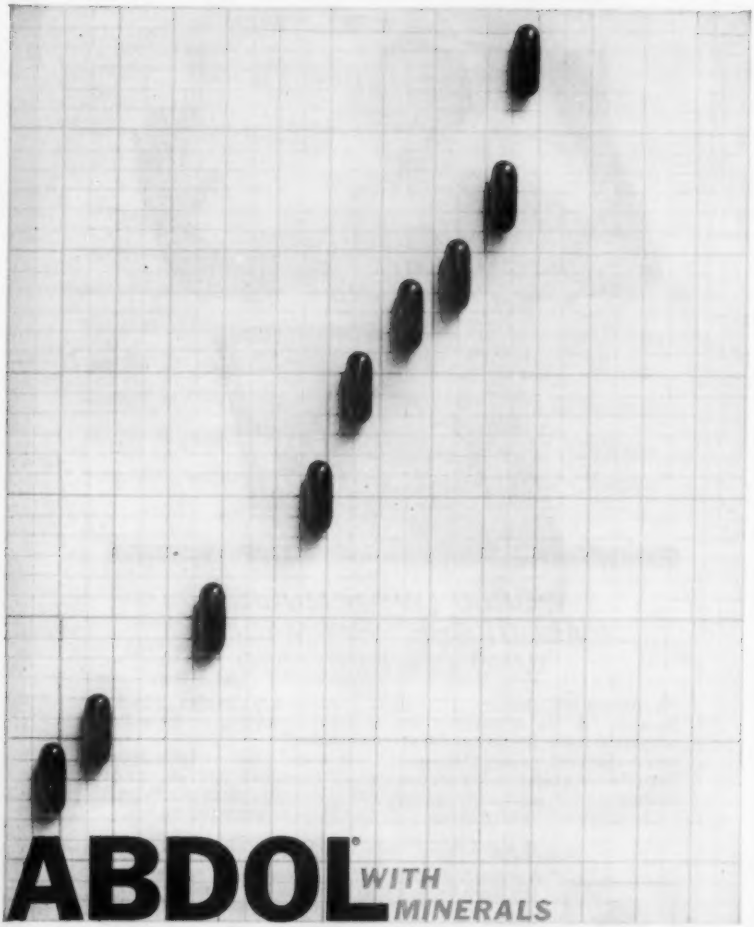

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E D I T O R I A L

GOVERNMENT PURCHASES OF DRUGS OVERSEAS

THE purchase of certain drugs by the Defense Department from foreign sources of supply is a new departure and one which should be widely condemned. Had these purchases been occasioned because the drugs in question were unavailable in the United States or because they were of better quality, we would have no objection. The decision to purchase them, however, was made solely on the basis of their lower price, and the Department of Defense attempted to justify its position by pointing out that they accomplished a considerable saving of government funds.

While the Department of Defense may know a great deal about military matters, we fear that they are sadly lacking in an understanding of basic economics and some of the facts of life; for example, why the American standard of living is higher than in those countries in which drugs can be bought more cheaply. Here, we wish to point out that it is not just pharmaceuticals that can be bought more cheaply overseas but practically every commodity that one could mention, particularly in the area of manufactured goods. We also wish to remind those who read this editorial that this new philosophy on the part of government of buying things where they can be purchased most cheaply threatens many industries other than the pharmaceutical industry and is a threat to American labor as well.

It is difficult to believe that those responsible for this decision are so naive as to believe that American manufacturers can produce and sell drugs as cheaply as can many foreign producers. They must surely know that one of the major costs in all productive effort is the cost of labor and that this cost is also involved in the cost of all materials, equipment, and machinery used by the drug manufacturer. One might also ask where, indeed, does the money come from which the Department of Defense uses to purchase drugs. Does it come from foreign loans awarded the United States by Italy and Denmark so that we may then have the purchasing power to buy their drugs

or does it come from these same companies and others who must price their products keeping in mind not only costs of production but our high rates of taxation as well?

It is ironic that the American taxpayer is today subsidizing many of the governments of the so-called free world and, yet, must sit quietly while his government who takes his money for this purpose then purchases products which it needs from his competitors overseas. If this philosophy were pushed to its logical conclusion, what our master-minds in government are suggesting is that we in the United States should compete in the world market with the elimination of all tariffs and all import duties. This we could do quite successfully if we in the United States are prepared to compensate American labor at the same rate as labor overseas and if we as the citizens of the United States are prepared to live on the same low standard as many of the people in other countries. This willingness to adopt the same standard of living and the same rate of pay would, of course, apply to all—business and labor—and, we may add, government officials and the military as well. We could even suggest that the Department of Defense might find it possible to obtain some excellent generals from both Italy and Denmark fully as competent as our own and willing to serve this country loyally at half the current rate for generals in the United States. Such a suggestion is just as logical as the purchase of drugs overseas and, we suspect, just about as acceptable to the Department of Defense as their recent activity is to our own pharmaceutical industry.

L. F. Tice

SEQUENCE OF AMINO-ACIDS IN PROTEINS AND POLYPEPTIDES

By V. D. Gupta * and Henry M. Burlage †

Introduction

IN recent years, protein chemistry has been extensively advanced from many different points of view; one of the most important is ascertaining the sequence of amino-acids in the various peptide chains. It is hoped in this way to gain knowledge in one of the most important fields. There must be some deep and fundamental reason why nature has chosen polypeptide chains as the building blocks for all forms of living matter—a reason which should become clear, only when we solve the mystery of the structure of the protein molecule. The knowledge of sequential arrangement of amino-acids began to appear in the early 1930's. Insofar as complete elucidation of sequence is concerned, the acquisition of such knowledge is not likely to be rapid in the near future. The results obtained so far are meagre. The difficulty and importance of attack on protein structure has been summarized by Sir Lawrence Bragg (1).

After the consideration of the amino-acid composition of the proteins, the question which naturally arises is that of the mode of linkage of the building material, i.e., amino-acids. It is now generally accepted that the predominant chemical linkage in proteins and polypeptides is the alpha-peptide linkage; that is, an amide linkage between amino and carboxyl groups of the amino-acids. However, other linkages are also present. The most important is the *disulfide linkage* which is present in proteins and polypeptides containing cystine (S-S) residues. This linkage may cross link two separate polypeptide chains or separate segments of a single chain. In the latter case, a closed loop results. Insulin molecules contain both types of disulfide linkages.

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Important Methods for the Elucidation of the Sequence of Amino-Acids in Proteins and Polypeptides

It would be worthwhile to review the methods used to find out the sequence of amino-acids in various proteins and polypeptides. We know that the essential linkage present in the peptide chain is the *amide* linkage, so that we have a free amino-group on one side (called N-terminal group) and a free carboxylic group on the other side of the peptide (called C-terminal group). The methods are divided into two classes; i.e., N-terminal and C-terminal—according to the side on which the attack is made. The third general class is that of the disulfide linkages.

N-terminal Methods

These methods involve either the formation of some derivative by a reaction between the free amino-group and some reagent or the separation of single amino-acid (which is present in the chain with free amino-group) by enzymatic hydrolysis. They can be further subdivided as follows:

(a) *DNFB Method:*—This method was developed by Sanger (2, 3) and Porter (4). In this method, 2-4-dinitrofluorobenzene (DNFB) reacts with the free amino-groups to form a dinitrophenyl derivative, which is highly colored in nature. The colored derivative can be easily separated by chromatographic analysis and confirmed by the technique of paper chromatography.

(b) *Para-iodophenyl Sulfonyl Method:*—Underfriend and Valick (5) preferred the use of this reagent in the form of its chloride. Unfortunately, it does not appear to react with the amino-groups of many amino-acids.

(c) *PTC Method:*—In this method, a phenylthiocarbamyl derivative instead of DNP-derivative is preferred. This method was originated by Edman (6, 7) and Conrat and Harris (8). This derivative on treatment with hydrochloric acid gives phenylthiohydantoin of the corresponding amino-acid, which is confirmed with the help of chromatography (9).

(d) *Thiocarbamate Method:*—This method was developed by Levy (10). A 2-thio-5-thiazolidone derivative of the amino-acid is prepared and confirmed with the help of chromatography (9).

(e) *Bromoacetate Method:*—This is a recent addition to the list of reagents used. For further details, refer to the work of Korman and Clarke (11, 12).

In all the foregoing methods, the hydrolysis is accomplished by an enzyme. The enzyme, amino-peptidase, specifically hydrolyzes the peptide bonds adjacent to free alpha amino-groups and liberates the free amino-acids derivatives.

C-terminal Methods

In this case, either free amino-acids or their derivatives (formed with carboxyl groups) of the amino-acids are liberated, as compared with the *N-terminal methods,* where the derivatives with the amino-groups are obtained.

(a) Chibnall and Rees (13) have described the reduction of the free carboxylic groups to amino-alcohols. This alcohol is then obtained free by hydrolysis and confirmed with the help of chromatography (12).

(b) Niu and Conrat (14) developed a method wherein they used hydrazine as the reagent and which converts all other amino-acids to hydrazides except the C-terminal amino-acids. These C-terminal amino-acids are then separated by enzymatic hydrolysis (which will specifically hydrolyze the bonds adjacent to the free carboxylic group; i.e., specific carboxy-peptidase) and confirmed with the help of chromatography (15).

Methods for Disulfide Linkages

Here, the S-S linkage is generally oxidized to cysteic acid (16, 17). This can be readily accomplished with the help of performic or peracetic acid. This oxidation breaks the S-S linkage and the amino-acids can be confirmed by the C-terminal and N-terminal methods.

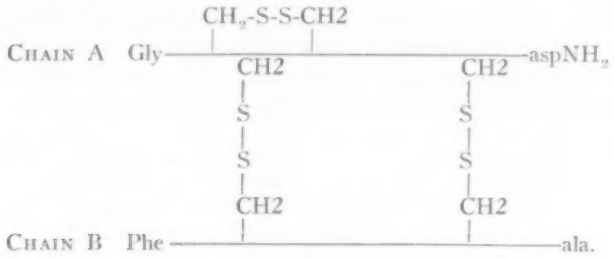
Sequence of Amino-Acids in Some Specific Hormones, Enzymes, and Miscellaneous Proteins

Hormones

Insulin—No other protein molecule of comparable size is understood in its structural details with as much certainty as insulin. Sanger

and his associates (18-22) have provided us with the complete amino-acids sequence of this well-defined protein. The insulin molecule was found to be composed of two peptide chains A and B which are joined by disulfide bonds. To break these bonds, Sanger used performic acid, which converts cystine(S-S) to cysteic acid molecules. The chains were then separated and their amino-acids sequence was determined with the help of the methods already described.

Chain A was shown to have 21 amino-acids with glycine as the N-terminal amino-acid (therefore, also called glycyl chain). Chain B contains 30 amino-acids with phenylalanine as the N-terminal amino-acid (also called phenylalanyl chain). The gross structure of insulin molecule can be represented as follows:—



Gross Structure of Insulin Molecule.

The abbreviations used are those suggested by Edsall and Brand (23) and the arrangement of amino-acids in the chains is according to Sanger (24).

It will be noted that the insulin molecule is characterized by the presence of a ring or loop involving "three half cystine" residues in Chain A. A similar structure has been established in the peptide hormones of the posterior pituitary gland. The presence of this unusual ring in several different proteins and polypeptides is a striking phenomenon, and it may bear special relation to the biological activities of these compounds. A single set of side chains is clearly not required for the physiological activity, as is evident from the amino-acids sequence in positions 8, 9, and 10 of the chain A, in the case of insulin molecules from various origins, *viz.*, pig, sheep, beef, whale, *etc.* In spite of the difference in the amino-acids at positions 8, 9, and 10 in chain A, there is no appreciable difference in their biological activities.

Amino-Acids at Position Nos. 8, 9, and 10 in Various Molecules—

Beef insulin	-ala-ser-val-
Swine insulin	-thr-ser-leu-
Sheep insulin	-ala-gly-val-
Horse insulin	-thr-gly-iso-
Whale insulin (25, 26)	-thr-ser-iso-

As indicated, the difference in the various insulin molecules is only in the amino-acids at positions 8, 9, and 10 of chain A. The rest of the amino-acids are exactly the same and in the same sequence. The complete structure of the insulin molecule can be written as follows:—

Chain A or Glycyl Chain (21 amino-acids)
Gly-iso-val-glu-glu(NH2)-cyS-cyS-8 to 10 (varies in each molecule; i.e., according to the origin, as given above)-cyS-ser-leu-tyr-glu(NH2)leu-gly-asp(NH2)-tyr-cyS-asp(NH2).

Chain B or Phenylalanyl Chain (30 amino-acids)
Phe-val-asp(NH2-glu(NH2)-his-leu-cyS-gly-ser-his-leu-val-glu-ala-leu-tyr-leu-val-cyS-gly-glu-arg-gly-phe-phe-tyr-tyr-pro-lys-ala.

For disulfide linkages, refer to the gross structure of insulin.

Oxytocin and Vasopressin—These hormones are from the posterior pituitary gland. Vigneaud and his associates (27-29) have worked out the sequence of amino-acids in them. The structure of oxytocin, at least, has been confirmed by synthesis by the same group of workers (27).

Structure of Oxytocin from Pig and Cow

CyS-tyr-*iso*-glu(NH2)-asp(NH2)-cyS-pro-*leu*-gly-NH2

Vasopressin from Pig

CyS-tyr-*phe*-glu(NH2)-asp(NH2)-cyS-pro-*arg*-gly-NH2

Vasopressin from Cow

CyS-tyr-*phe*-glu(NH2)-asp(NH2)-cyS-pro-*lys*-gly-NH2

The small structural differences (See the amino-acids which have been italicized), however, permit large differences in biological activities. In the insulin molecule, the small structural differences do not result in appreciable differences in biological activities.

Glucagon—This pancreatic hormone acts as hyperglycemic and glycogenolytic. Bromer and his associates (30) have reported the sequence of amino-acids as follows:—

His-ser-glu(NH2)-gly-thr-phe-thr-ser-asp-tyr-
ser-lys-tyr-leu-asp-ser-arg-arg-ala-glu(NH2)-
asp-phe-val-glu(NH2)-tyr-leu-met-asp(NH2)-thr.

Melanocyte Stimulating Hormone (MSH)—This is another hormone from the pituitary gland, which has been purified and studied in detail. The sequence of amino-acids has been reported by two group of workers: Harris and his associates (31-34) and Geshwind and his co-workers (35-37).

The following amino-acids sequences have been reported:

Beta MSH from Human (38)

H-ala-glu-lys-lys-asp-glu-gly-pro-tyr-arg-met-
glu-his-phe-arg-tyr-gly-ser-pro-pro-lys-asp-OH.

Alpha MSH from the Pig (31)

CH3CO-ser-tyr-ser-met-glu-his-phe-arg-tyr-gly-
lys-pro-val-NH2.

Beta MSH from the Pig (35)

H-asp-glu-gly-pro-tyr-lys-met-glu-his-phe-arg-
tyr-gly-ser-pro-pro-lys-asp-OH.

Beta MSH from the Ox (37)

H-asp-ser-gly-pro-tyr-lys-met-glu-his-phe-arg-
tyr-gly-ser-pro-pro-lys-asp-OH.

Adrenocorticotropic Hormone (ACTH)—This is one of the most important hormones from the adrenal cortex. The complete sequence of amino-acids of this hormone from the pig and sheep have been reported by three groups of workers; i.e., Bell and his associates

(39), Shephered and his co-workers (40), and Li and his associates (41).

The following groups of workers have also made some contributions: Harris and his associates (42) and Woolley and Merrifield (43).

All of the workers have shown that the ACTH from the pig and sheep have almost identical structures with only minor differences.

Alpha Corticotropin from the Sheep (41, 42, 44, 45)

Ser-tyr-ser-met-glu-his-phe-arg-tyr-gly-lys-pro-val-gly-lys-lys-arg-arg-pro-val-lys-val-tyr-pro-ala-gly-glu-asp-asp-glu-ala-ser-glu-ala-phe-pro-leu-glu-phe.

Alpha Corticotropin from the Pig (46)

Ser-tyr-ser-met-glu-his-phe-arg-tyr-gly-lys-pro-val-gly-lys-lys-arg-arg-pro-val-lys-val-tyr-pro-gly-ala-glu-asp-asp-glu-leu-ala-glu-ala-phe-pro-leu-glu-phe.

Beta Corticotropin from the Pig (39, 40)

Ser-tyr-ser-met-glu-his-phe-arg-tyr-gly-lys-pro-val-gly-lys-lys-arg-arg-pro-val-lys-val-tyr-pro-gly-ala-glu-asp-asp-glu-leu-ala-glu-ala-phe-pro-leu-glu-phe.

It has been shown that many major chemical changes can be made in the structure of ACTH without affecting the biological specificity (43).

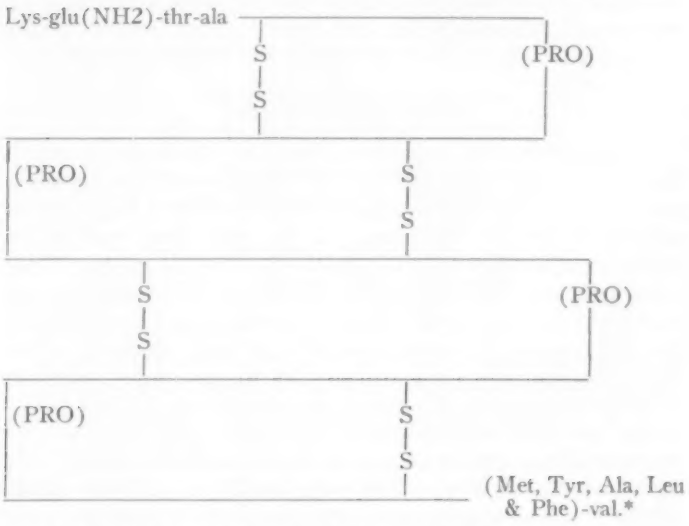
Enzymes

Ribonuclease—Anfesin and his associates (47, 48) have worked on this protein and have reported its gross structure. Complete sequence of amino-acids has not been elucidated as yet. Anfensin has reported that this enzyme is a single chain of 121 amino-acids. In the opinion of other workers, this single chain contains 124 amino-acids. However, Anfensin and his associates are the only investigators who have given us the gross structure of this protein.

According to the latest report by Hirs (49), all of the five segments have been purified and the sequence of amino-acids in these segments is being studied.

The single chain, however, has four folds which are due to the presence of four proline molecules. These proline residues act as a center of direction reversal in this chain (50).

Generalized Gross Structure of Ribonuclease (48)



* Order of Met, Tyr, Ala, Leu and Phe not determined.

Physical and chemical studies indicate that the chain is cross linked through four disulfide bridges. If the enzyme is oxidized with performic acid, these bridges are broken and each molecule of cystine is converted to two molecules of cysteic acid. This acid can be determined by simple titration and the number of disulfide linkages present may be calculated.

Taka Amylase A (TAA)—This is an enzyme which hydrolyzes starch molecules and studies on it have been made by Japanese workers (51, 52).

Tsugita (51) has worked on this protein and has published many papers on his studies. The sequence of amino-acids in this enzyme is still unknown. From the various experiments, Tsugita has con-

cluded that the amino-acids sequence in the N-terminal region of TAA is:—

Ala-gly-asp-glu-ser-ala-leu-thr.

The determination of C-terminal amino-acids of this complex enzyme was undertaken by Ikenaka (52), who has found that each molecule of TAA contains Gly, Ala, and Ser in the C-terminal region.

Complete sequence of amino-acids in this protein is still unknown.

Miscellaneous Proteins

Hemoglobin—The most interesting thing about this protein is the number of free SH groups present. This puzzled many groups of workers for a long period. First of all, Ingram (53) suggested the presence of four free SH groups. It was later shown that the denatured molecule of the protein contains six free SH groups. Then, it was postulated that two members of the free SH groups were unavailable in the native protein molecule; hence, actually there were six free SH groups. This explanation was still under discussion, when Murayama (54) solved the problem, and his work was accepted by all groups of workers. He reported that Hemoglobin A and S (Adult normal and sickle cell hemoglobin respectively) have three and two titratable SH groups respectively at 38° C. and that both have four titratable groups at 0° C. The titration was done with silver nitrate solution. It is now quite certain that four is the correct number of SH groups per molecule of hemoglobin.

The work of Rhinesmith and his associates (55, 56) has shown valine to be the N-terminal residue in this complex protein. As there are four polypeptide chains per molecule of hemoglobin, so the number of N-terminal amino-acids; i.e. valine, is four.

Huisman and Dozy (57, 58) have done some work on the C-terminal amino-acids of this protein. This, however, still remains unconfirmed and is a challenge to the workers in the field.

How a small difference in the amino-acids can cause a great change in the biological specificity is apparent in the case of hemoglobin. Samples of hemoglobin A and S were analyzed under comparable conditions (59, 60) and the results indicated that hemoglobin S contains less leucine and more serine and possibly less valine and more threonine than in the case of hemoglobin A. Ingram (53, 61) examined the tryptic hydrolysates of hemoglobin A and S. He has

pointed out that each of these contains a unique segment, in which a valine residue in hemoglobin S replaces a glutamic residue in hemoglobin A.

Later, it was shown that the same segment is affected in the case of abnormal hemoglobin, which indicates the presence of lysine or arginine residue instead of glutamic in the case of hemoglobin A.

Structure of the Unique Segment—

Hemoglobin A (Adult normal)
His-val-leu-leu-thr-pro-*glu*-glu-lys.

Hemoglobin S (Sickle cell)
His-val-leu-leu-thr-pro-*val*-glu-lys.

Abnormal Hemoglobin
His-val-leu-leu-thr-pro-*lys*-glu-lys.
or *arg*

These experiments have provided the first conclusive demonstration of a chemical difference between the proteins and represents a significant advance in the elucidation of their differences.

Silk Fibroin—The fibroin *Bombyx mori* has attracted the attention of scientists and technologists for many years. This is mainly because of its greater commercial importance and also due to the fact that the literature concerning its physical and chemical properties is abundant.

Most of the work on the sequence of amino-acids in this protein has been done by two groups of workers: Meyer and his associates (62), and Lucas and his associates (63-65).

This protein has been studied after chymotryptic hydrolysis. The hydrolysis gives a granular precipitate (60 per cent of the total protein) called *chymotryptic precipitate* or simply *CTP*. The remaining 40 per cent of the *chymotryptic liquor* is called *CTL*. The *CTP* part is the crystalline part of the fiber while *CTL* is the amorphous part of the fiber.

It was in 1940 when Meyer and his associates suggested the following sequence of amino-acids in this protein:—

Gly-arg-gly-tyr-(gly-arg-gly-arg-gly-ser-gly-arg-gly-arg-gly-arg)gly-tyr-arg-gly.

According to Meyer and his associates, the portion within the parentheses forms the crystalline part of the fiber (*CTP*), while the rest forms the amorphous part; i.e., *CTL*.

This sequence, however, is not consistent with the recent research by Lucas and his associates (63-65) and other workers. All evidence in the case of *CTP* portion is that it consists of units of hexapeptide repeated eight times, together with an introductory sequence beginning with glycine and a terminal sequence ending with tyrosine. In order to allow the possibility of occasional sequences longer or shorter than six occuring, it was suggested that the repeating unit should be expressed as Ser-gly-(ala-gly)n, where n is usually two and always has a mean value of two.

Sequence of Amino-Acids in the Crystalline (CTP) Portion of Silk Fibroin—

Gly-ala-gly-ala-gly[ser-gly(ala-gly)n]8-ser-gly-
ala-ala-gly-tyr

where n is usually two and always has a mean value of two.

The amorphous portion (*CTL*) still remains to be studied. This also is being extensively studied by Lucas and his associates. After these studies are reported, something conclusive concerning the complete amino-acids sequence in this structural protein might be in order.

Discussion and Conclusion

Today, more than one thousand proteins are known and it is interesting to note that most of them are found in the living matter. Almost all the enzymes and hormones are protein in nature. Proteins are building units of our bodies. It is, therefore, very important to work out the sequence of amino-acids of such an important group of compounds.

It is only by elucidating the sequence of amino-acids of these proteins and polypeptides that we can understand (a) Why nature has chosen this complex group of compounds as the building material for all the living matter; (b) The methods of synthesis of enzymes, hormones, and many other important compounds; (c) Why a minor change in the amino-acids, sometimes, causes a great change in the biological specificity; (d) Why it is possible to make major chemical changes in the case of insulin without affecting its biological specificity;

and (e) The mechanism of action of hormones, enzymes, and other biologically active proteins and polypeptides.

It is felt that biochemists and other scientists in the allied fields should divert greater attention to this important field. It is only by more detailed studies that it may be possible to say "How the Living Organism Operates."

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PHOSPHORAMIDES IN THE MANAGEMENT OF LYMPHOMAS AND RETICULOSES

By John R. Sampey *

A RECENT review (23) showed that phosphoramides are among the most promising of the newer chemotherapeutic agents in the control of acute and chronic leukemias. The present study demonstrates that these chemicals have been even more extensively employed in the management of a number of malignant lymphomas and reticuloses. Triethylene thiophosphoramide (Thio-TEPA) again dominated the other phosphoramides in its antineoplastic action.

Hodgkin's Disease

A survey of two dozen clinical reports on the use of phosphoramides in the treatment of Hodgkin's disease indicates the importance of these chemicals in this reticulosis. Leonard and associates (12), described six complete and five partial remissions in 11 patients following Thio-TEPA therapy. Stradyn and Tseplite (27) employed this same drug in 15 cases to induce some clinical improvement. Ultmann *et al.* (31) in 1957 reported five remissions lasting to two months in 13 cases of Hodgkin's disease and, the next year (32), they noted three excellent and six partial remissions in 13 cases under Thio-TEPA management. Bateman (3) used both Thio-TEPA and N(3-oxypentamethylene)-N′N′′-diethylene phosphoramide to secure regression of tumors in three of 13 patients. Zubrod conducted two appraisals in a quantitative comparison of nitrogen mustards and phosphoramides in cancer in man: in 1958 (36), he concluded that HN2 was significantly better than Thio-TEPA in Hodgkin's disease and, in 1960 (37), he and 18 others stated that, in 11 patients, five showed improvement on Thio-TEPA and nine of nine were benefited by HN2.

Wright and associates carried out two clinical trials on phosphoramide therapy of Hodgkin's disease. In 1957 (34), they recorded one complete remission for two years and two slight regressions in five patients on Thio-TEPA. The following year (35), they observed

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three regressions in five patients, lasting to 20 months. Leukopenia developed in 59 of their 94 patients with various neoplasms, but they considered the drug safe if the blood counts were watched. Limited response to Thio-TEPA therapy was reported by Koyama and Toknyama (10), and Mininni (17) in two of three cases each of Hodgkin's disease; Shay *et al.* (25) observed limited response in one of three cases, while Leone *et al.* (13) classed their reaction in three patients as poor. Farber *et al.* (7) induced a 27 months' remission in one of two cases on Thio-TEPA, and Winkler *et al.* (33) noted some improvement in two cases. Magalini (15) caused a fair remission in one patient who was alive at the time of writing after two months of treatment. Hunter (9) induced a fair response in one patient on Thio-TEPA, and Smith *et al.* (26) reported clinical improvement in children undergoing similar therapy.

Sykes *et al.* (28) described two good and one fair objective response in four cases of Hodgkin's disease after treatment with triethylene phosphoramide (TEPA) but only a two weeks' response in one case on diethylenephosphoramide (DEPA). Bernard and Deltour (4) noted some prolongation of life in a patient under TEPA treatment.

The new cytostatic drug, N,N-bis-(β-chlorethyl)-N',o-propylene phosphoric acid ester diamide, with the trade names of Endoxan, Cytoxan, and cyclophamide, has been tested on Hodgkin's disease by several investigators. Cramblett (5) reported two complete remissions, lasting three and four and one-half months each. Saitmacher (22) employed x-rays and Endoxan to induce smaller tumors and better general condition in two of three patients, but the third patient was made worse. Kühböck *et al.* (11) indicated some favorable response in Hodgkin's disease and other reticuloses after Endoxan therapy.

Lymphosarcoma

More than a dozen clinical trials have been made on the cytostatic action of phosphoramides in lymphosarcoma. Ultmann and associates conducted two studies on the action of Thio-TEPA: in 1957 (31), they reported a partial remission of six months in one out of five patients with lymphocytic lymphosarcoma, and a partial remission in one of two patients with giant follicular lymphosarcoma on a combination of Thio-TEPA and cortisone. The next year (32), they

recorded one excellent remission and nine partial in 30 cases of lymphosarcoma on Thio-TEPA. Alpert (1) treated 12 patients with this phosphoramide, and observed regression in one lymphosarcoma. Wright *et al.* (35) described a two weeks' response in one of four cases, and Smith *et al* (26) obtained brief responses in five cases of lymphosarcoma. Leone *et al.* (13) classified the reaction of three lymphosarcoma cases as good, following Thio-TEPA administration. Edwards and Rathmell (6) induced nine months' survival in one patient on this drug, and they combined it with prednisone and x-rays to secure a three months' good response in another patient. Winkler (33) observed little effect of phosphoramide in one patient and Arrau *et al* (2) and Koyama and Toknyama (10) reported no response in single cases on Thio-TEPA therapy. Stradyn and Tseplite (27) found some response in three patients on this drug. Sykes *et al.* (28) chronicled one five months' remission in three patients on TEPA, but no response in two patients given DEPA. Endoxan was employed by Saitmacher (22) to induce some clinical improvement in two of three cases of lymphosarcoma. Papac *et al.* (19) preferred cyclophosphoramide to mannitol mustard in the management of three patients on each drug, and Kühböck *et al.* (11) reported some regressions with Endoxan.

Reticulum-Cell Sarcoma

Thio-TEPA has also dominated the therapy of reticulum-cell sarcoma. Ultmann *et al.* (31) observed two partial regressions to 12 months in 23 patients, and Koyama and Toknyama (10) described objective response in five of 12 patients. Leone *et al.* (13) caused excellent remissions in four patients with reticulum-cell sarcoma, and Leonard *et al.* (12) secured no response in two reticulosarcomas, but three of three lymphoid reticuloses had regressions, and one of three reticulo cell reticuloses showed improvement on Thio-TEPA. Sykes *et al.* (28) noted a two weeks' regression in one reticulum-cell sarcoma on this chemical, and Winkler *et al.* (33) reported little response in another case. Both Mininni (17) and Arrau *et al.* (2) found no improvement in single cases of reticulum-cell sarcoma after Thio-TEPA administration. Takahashi *et al.* (30) employed this drug to induce clinical improvement in both reticulosarcoma and acute leukemia, and they found that metallochlorphyllin protected against leukopenic action of Thio-TEPA. Rundles *et al.* (21) described a

surprising resolution of reticulum-cell sarcomas in four patients after administration of Endoxan.

Multiple Myeloma

Shay and coworkers (25) reported that one multiple myeloma had a good subjective response to Thio-TEPA, but Maney and Shen (16) stated that one patient developed thrombocytopenia on the drug, and Wright *et al.* (35) described the steady downhill course of another patient under this therapy. Oxapentamethylenediethylene thiophosphoramide (OPSPA) induced objective tumor regression in one of two patients with multiple myeloma under persistent therapy according to McIver *et al.* (14) while intermittent doses induced objective regression in three of four patients with myeloma and lymphoma.

Mycosis Fungoides

Sykes *et al.* (28) reported one good and one fair objective response in two patients with mycosis fungoides who were placed on TEPA therapy, but Goldman (8) recorded no change in a patient after local injection of TEPA. Seiter (24) observed no benefit in a patient after using Thio-TEPA and Bayer 3231. Rundles *et al.* (21) described the action of Endoxan in mycosis fungoides as similar to that of other nitrogen mustards.

Polycythemia Vera

Leonard *et al.* (12) treated 14 patients with polycythemia vera with Thio-TEPA and noted that all responded for as long as 14 months, with two alive at the time of reporting.

Miscellaneous Lymphomas

Olsen (18) recorded five objective and seven subjective improvements in 11 patients with advanced lymphomas who were administered Thio-TEPA, but he described leukopenia in 10 of the 11. He also observed that one patient with Ewing's tumor showed some subjective response. McIver *et al.* (14) remarked on the objective tumor regression by persistent OPSPA therapy in three of six patients with malignant lymphomas, while three of four on intermittent doses had objective regressions. Cramblett (5) noted that one of two lymphoma patients experienced dramatic benefit for five months after Endoxan

therapy, but that two with Wilm's tumors failed to respond. Pinkel (20) failed to obtain any objective regression in two patients with Wilm's tumors and one with Ewing's tumor following cyclophamide therapy.

Acknowledgments

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PLURONICS AS A SUPPOSITORY BASE

By Jere Neville * and William B. Swafford **

EVEN during the time of Hippocrates (460-377 B. C.), suppositories were used to administer medication. The first official suppository base in this country was a cocoa butter base introduced into U.S.P. V. From hand molding processes to crude types of molds the preparation of suppositories in the pharmacy laboratory has progressed to scientifically prepared metal molds which produce uniform suppositories with no difficulty of manipulation (1).

There is a definite need for systemic medications in the suppository form. For babies and small children, for unconscious or anesthesized patients, and for nauseated patients, the ease of administration of drugs by the suppository method is unsurpassed. There are no needles and syringes to be sterilized, no pain or fear of pain as with an injection, and no special skill is required for administration.

With the increase in the number of vaccines and other drugs requiring refrigeration, there is a definite advantage to a suppository base that will maintain its stability at room temperature.

The Pluronics (2), a new series of nonionic surface-active agents with the structure, $HO(C_2H_4O)_a(C_3H_6O)_b(C_2H_4O)_cH$, are believed to fulfill all the requirements for the ideal suppository base. The development of the Pluronics was based on the observation that, as the molecular weight of a polypropylene glycol is increased, the glycol passes from a water-soluble to a relatively water-insoluble material. At higher molecular weights, it is sufficiently water-insoluble to be a suitable hydrophobic base for nonionic surface-active agents. Surface activity is obtained by condensing ethylene oxide on the polyoxypropylene base.

There are four compositions of the Pluronics available commercially at the present. They are: Pluronics L44, L62, L64, and F68.

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They have practically no odor or taste which makes them ideal as an experimental research product for use in the preparation of syrups, waters, ointments, and suppositories. The most important requirements for the use of Pluronics in suppositories are: there should be no toxic side effects; they should melt rapidly in body fluids; they should require no refrigeration during storage; and the firmness of the suppository should not be affected by the addition of the medicinal ingredient.

Extensive study has been made on the effect of Pluronics on guinea pigs, rats, dogs, and rabbits. Oral administration of large doses of F68 does not produce toxic effects (2). The Pluronics do form toxic products with some of the phenolic compounds. In performing research on Pluronics as a base for aspirin suppositories, no information was located as to whether a toxic product resulted when the two are mixed.

Upon observation of the suppository with a Pluronic base, the conclusion has been reached that they may be stored without refrigeration in a closed glass container at room temperature. Some suppositories have been stored for a month and they show no deterioration.

The experimental medicinal agent (aspirin) affected the firmness of the experimental base. The formula had to be adjusted to soften the original base after the addition of aspirin powder. In the final aspirin suppository, more than 50 per cent of the liquid (L44) had to be used to produce a satisfactory finished product.

The first satisfactory base produced was:

Pluronic F68 .....1.5 Gm.
Pluronic L44 .....1.0 ml.

It was prepared by placing the solid (F68) and the liquid (L44) in a beaker and melting on a water bath. The beaker was removed, the mixture stirred thoroughly and poured into the mold. After the suppository had hardened, it was removed from the mold. This base proved to be most satisfactory because it had a smooth, greasy feeling to the hand and its disintegration period was about 17 minutes.

The problem of finding the right combination of Pluronics and aspirin was now undertaken. A number of combinations were tried and the following formula found to give a satisfactory aspirin suppository in a Pluronics base:

Pluronic F68 .....6.00 Gm.
Pluronic L44 .....7.00 ml.
Aspirin .....1.02 Gm.

To make 4-5 gr. suppositories,
each weighing 2.36 Gms.

The suppository was prepared by placing the Pluronics in a beaker and heating on a water bath until melted. The aspirin was then added and the mixture was stirred until a uniform solution was obtained. The solution was then placed in the mold and allowed to cool. It was ideal because it had a smooth, greasy feeling to the hand and the distintegration period was about 25 minutes in water at body temperature. The period required for setting up is not much increased over the first base without the aspirin included.

Since no means were available at this time to test the rate of absorption of the Pluronic base suppository, no comparison can be made between it and the most widely used base but, due to the chemical similarity between polyethylene glycol and Pluronic, it can be assumed that there will be a similar rate of absorption. Using the blood level obtained from the administration of the oral tablets as 100%, the average per cent absorption from the polyethylene glycol was 93.1%, from cacao butter was 65.5%, and from glycerinated gelatin was 52.9% (3).

In conclusion, this experimentation has arrived at a satisfactory Pluronic base aspirin suppository and has tested it as thoroughly as possible with the facilities available. However, it should not be taken for granted that they would not produce toxic side effects and, before any further attempt at development is considered, they should be tested thoroughly in some of the experimental animals.

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BOOK REVIEWS

Subsidia Pharmaceutica and Index Nominum 1960. Editors: Scientific Center of the Swiss Society of Pharmacists. 470 pp. Publishers: Swiss Society of Pharmacists (Selbstverlag des Schweizerischen Apotheker Vereins), Zürich, 1960. Price: 35.—Swiss Francs (Approx. $9.00).

In continuation of their established policy (see also book reviews, this journal—March 1958 and May 1959), the Scientific Center of the Swiss Society of Pharmacists (SSPh) has published a new *Index Nominum 1960* and a supplement to the *Subsidia Pharmaceutica*.

The *Index Nominum 1960* represents a complete revision of the editions of 1958 and 1959 and additional new product information, thus comprising one new alphabetical index of about 470 pages. It follows the same arrangement as in the previous editions, giving an alphabetical listing of names, chemical formulas, indications for use, and the names of the proprietary products. It is one of the most useful reference books that should be on the shelf of pharmacists who seek information on European and American products.

The supplement to the *Subsidia Pharmaceutica* consists of additions to three different divisions:

1. The addition to the "Therapeutische Stoffklassen" deals with an article on antihistamines, giving information on their chemistry, therapeutic effectiveness, etc.

2. To the division of "Galenische Präparate" is added a monograph on: Unguentum Hydrocortisoni 1%.

3. Lastly, the division of "Pharmazeutische Kleinapparaturen" is further augmented by three separate articles:

   a. A description of a special sterilization apparatus by filtration.

   b. A description of a movable drying and sterilization cabinet.

   c. New suppository molds.

The SSPh is rendering a great service not only to its members who will greatly profit from these publications but also to the pharmaceutical profession at large. This reviewer has used the *Index Nominum* constantly in interpreting foreign prescriptions with particular reference to trade names not commonly known in this country.

ELSA EHRENSTEIN

Methoden der Organischen Chemie (Houben-Weyl). Fourth Edition. Volume 5, Part 4. Halogenverbindungen. Edited by Eugen Müller. 894 pages, with 11 illustrations. Georg Thieme Verlag, Stuttgart, 1960. DM 180 (approximately $43).

Part 4 of Volume 5 of the Houben-Weyl series deals with the preparation of bromine- and iodine-containing organic compounds and with the reactivity and transformation of organic chlorine, bromine, and iodine derivatives.

Under the general heading of Preparation of Bromine-containing Compounds, the following subjects are discussed: (1) Survey of Brominating Agents (25 pages); (2) Introduction of Bromine by Addition of Bromine or Bromine-containing Compounds to Unsaturated Systems (115 pages); (3) Introduction of Bromine by Exchange with Hydrogen (201 pages); (4) Introduction of Bromine by Exchange with Other Atoms or Groups (113 pages); (5) Preparation of Bromine Derivatives by Special Reactions (51 pages).

A section on Preparation of Iodine-containing Compounds is similar in arrangement to the preceding; this section contains a total of 161 pages.

A section entitled Reactivity and Transformation of Chlorine, Bromine and Iodine Compounds contains 96 pages of subject matter.

The literature coverage for this particular book extends to the beginning of 1958; the comprehensiveness of it may be judged by the fact that 76 pages are required for the author index, each page of which contains about 125 entries.

Another outstanding contribution to the literature of organic chemistry has been made by the editors and authors of Houben-Weyl.

ARTHUR OSOL

An Introduction to Pharmacology. J. J. Lewis. xii + 826 pp. Williams and Wilkens Co., Baltimore, Md., 1960. Price: $11.00.

The announcement of another book about such a subject as pharmacology normally does not create much furor. The apathy of educators, scientists, and many others is abruptly shattered, however, by the examination of this text.

In the case of this book, the somewhat hackneyed expression "new" regains its original significance and meaning. This is a unique and distinctly different presentation of the subject of pharmacology. The organization of the material reflects quite obviously the extensive background, abilities, and experience of the author. The information is discussed comprehensively, precisely, and conclusively in a logical sequence. The accepted concepts have been given preference and the controversial arguments avoided. Therapeutic agents are mentioned by generic, chemical, and selected proprietary names, without mention of manufacturers.

The general format of this book follows a sequence in which a brief anatomical-physiological and pathological introduction to each class of drugs initiates the discussion. Therapeutic applications, mode of action, dosages, toxicity, and side-effects are included for each important agent.

The action of the drugs in the body is described in the terminology of the biochemist. Human and animal applications are inspected and, for the benefit of laboratory personnel, testing procedures are outlined. Since the pharmacist must be familiar with all these areas, this book is particularly adaptable to his needs.

Introductory portions of the text condense the essence of pharmacodynamics into a brief but understandable chapter, after which administration, absorption, metabolism, and excretion are considered. The basic statistical concepts pertinent to evaluating drug action are illustrated and explained.

The author is to be commended for his efforts in the preparation of this book, which conveys in its pages so much, to so many, so clearly. The value of the information contained in this work and the up-to-date quality of the material fully justify the addition of this book to a library.

THOMAS H. F. SMITH

Synthetic Analgesics, Part I Diphenylpropylamines. Paul A. J. Janssen. 183 pp. Pergamon Press, New York, N. Y., 1960. Price: $7.50.

Dr. Jannsen describes and discusses the methods of synthesis and the physical and chemical properties, as well as the analgesic activity of diphenylpropylamines. Other pharmacological properties of these compounds are also mentioned.

A very critical and systematic approach has been taken in this review. The facts are presented in the form of tables so one can readily judge the presence or absence of agreement among various authors. The relationship between chemical structure and biological activity is discussed as well as presented in these tables.

No one who is interested in the field of analgesia and who desires a comprehensive and informative reference to this group of drugs should be without this series.

BENJAMIN WEISS

The Neurochemistry of Nucleotides and Amino Acids. R. O. Brady and O. B. Tower, Ed. xii + 292 pp. John Wiley and Sons, Inc., New York 16, N. Y., 1960. Price: $10.00.

This book represents the first detailed, comprehensive coverage of current knowledge of the roles and metabolism of nucleotides and amino acids in the central nervous system. This text is the culmination of a symposium on this subject held in 1958 and is distinguished by contributions of some of the leading investigators in various phases of the subjects covered.

Each chapter is devoted to a class of nucleotides or amino acids and the chemistry, biochemistry, and physiology of these compounds are discussed in addition to the recent findings of the investigators attending the symposium.

The contents have been designed to serve as a reference and source book for students and investigators contemplating exploration of problems in the neurological sciences.

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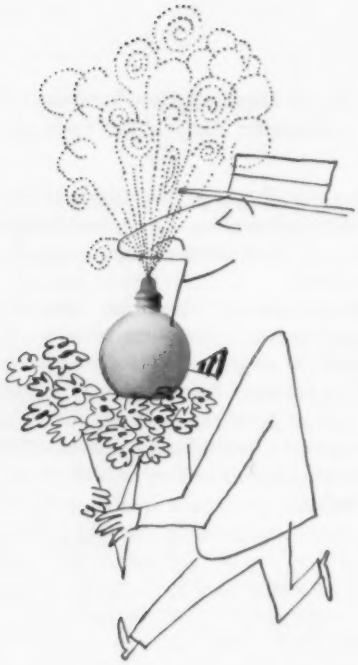

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